

Single-cell RNA-seq (scRNA-seq) Methanol fixation

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 An abbreviated version of this protocol was published in Science in Oct 2020

Gene regulatory networks controlling vertebrate retinal regeneration

DOI: 10.1126/science.abb8598

Detailed protocol

Hi, Atsuko,

Bellow is the our protocol for methanol fixation of mouse retinal cells. We basically follow the 10x protocol

:https://assets.ctfassets.net/an68im79xiti/7rsw40AVqX3ZXwll7MDj85/fb7ac4e1b324827f5b738ade5a02b650/CG000136_Demonstrated_Protocol_MethanolFi

Methanol Fixation for scRNA-Seq for mouse retinal cells

- Centrifuge cells 300 xg at 4°C for 5 min
- Remove supernatant, add 1 ml 4°C D-PBS (Thermo-Fischer Scientific, 14190144), resuspend cells by pipetting gently
- Centrifuge cells 300 xg at 4°C for 5 min
- Remove supernatant, add 100 ml 4°C D-PBS, resuspend cells by pipetting
- Add 900 ml -20°C 100% methanol (Sigma-Aldrich 34860-100ML-R) dropwise, while gentle mixing slowly (prevents cells clumping),
- Leave on ice for 15 min, then place in -80°C (sample will remain as liquid, and can be moved or shipped on dry ice)

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Hoang, T. (2022). Single-cell RNA-seq (scRNA-seq) Methanol fixation. Bio-protocol Preprint. bio-protocol.org/prep1539.
2. Hoang, T., Wang, J., Boyd, P., Wang, F., Santiago, C., Jiang, L., Yoo, S., Lahne, M., Todd, L. J., Jia, M., Saez, C., Keuthan, C., Palazzo, I., Squires, N., Campbell, W. A., Rajaii, F., Parayil, T., Trinh, V., Kim, D. W., Wang, G., Campbell, L. J., Ash, J., Fischer, A. J., Hyde, D. R., Qian, J. and Blackshaw, S. (2020). Gene regulatory networks controlling vertebrate retinal regeneration. Science. DOI: [10.1126/science.abb8598](https://doi.org/10.1126/science.abb8598)

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